

Impact of systemic therapy on circulating leukocyte populations in patients with metastatic breast cancer

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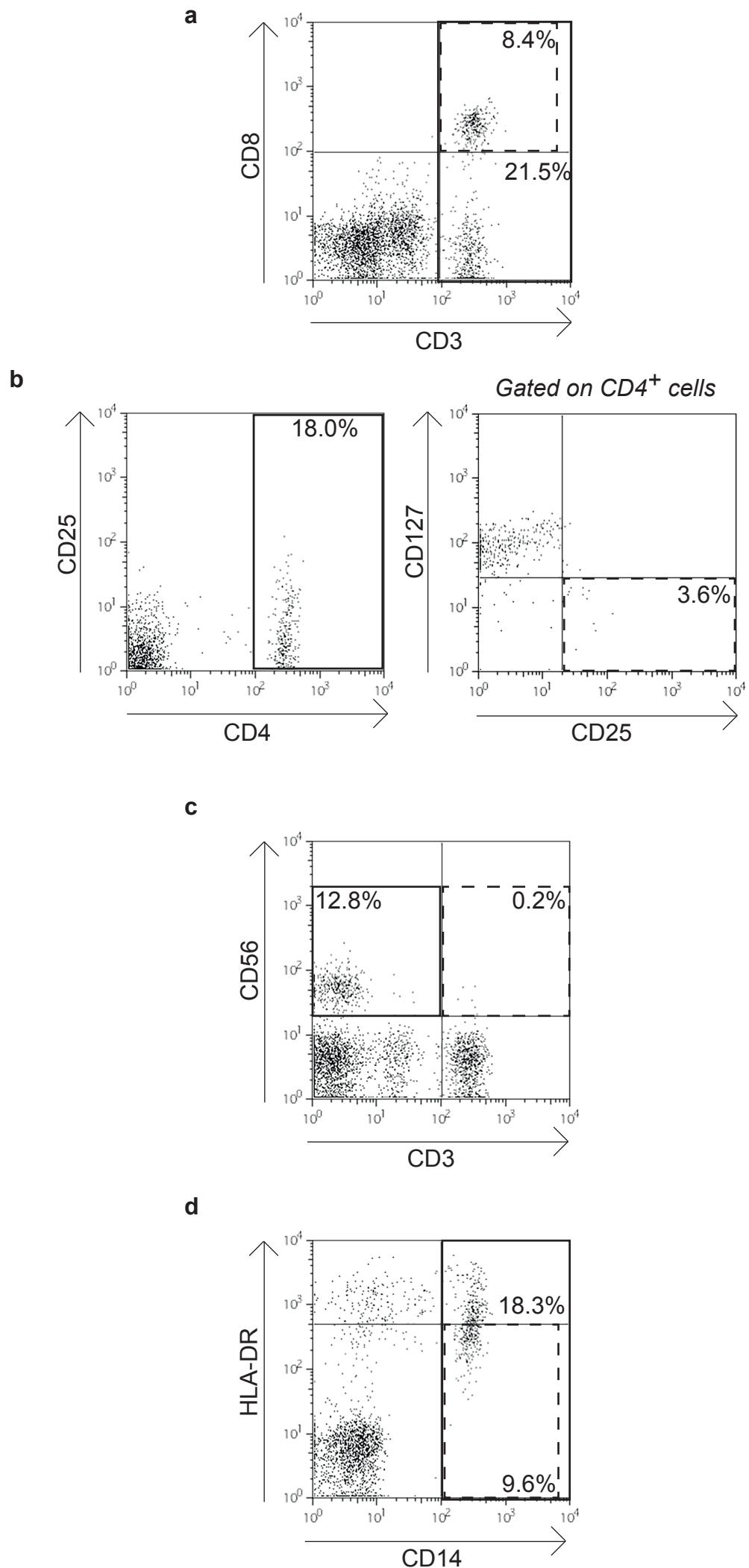
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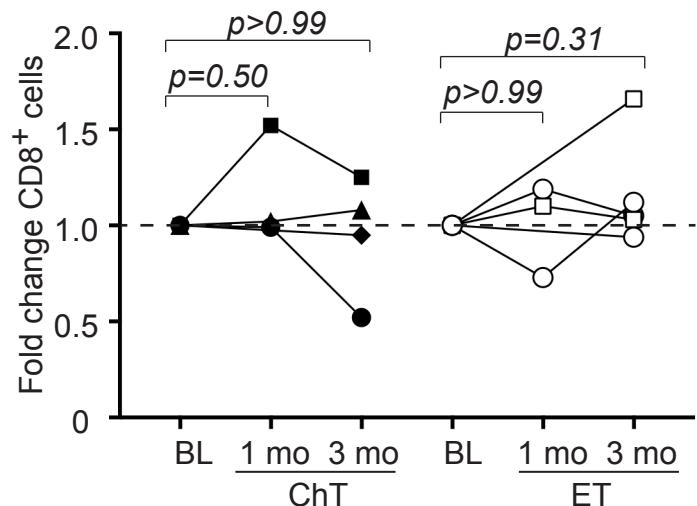
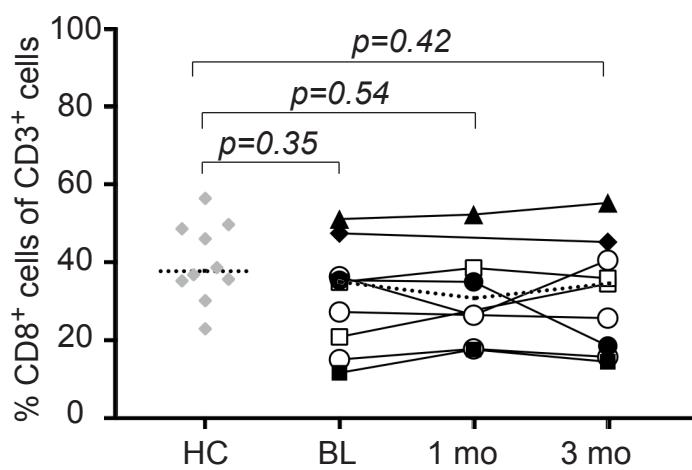
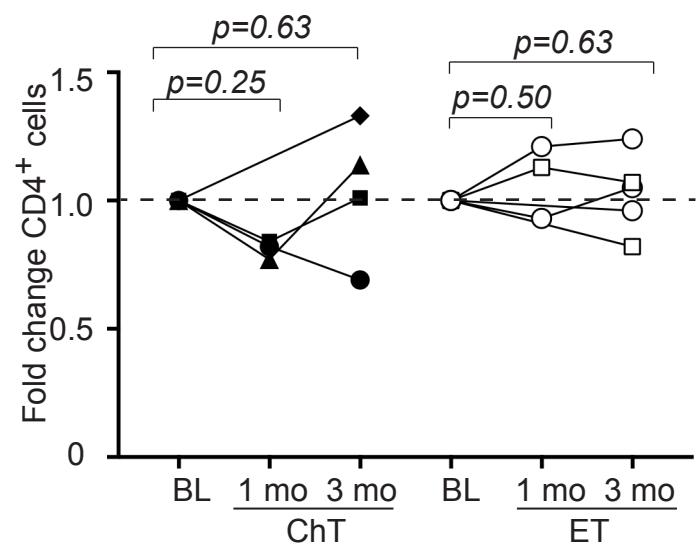
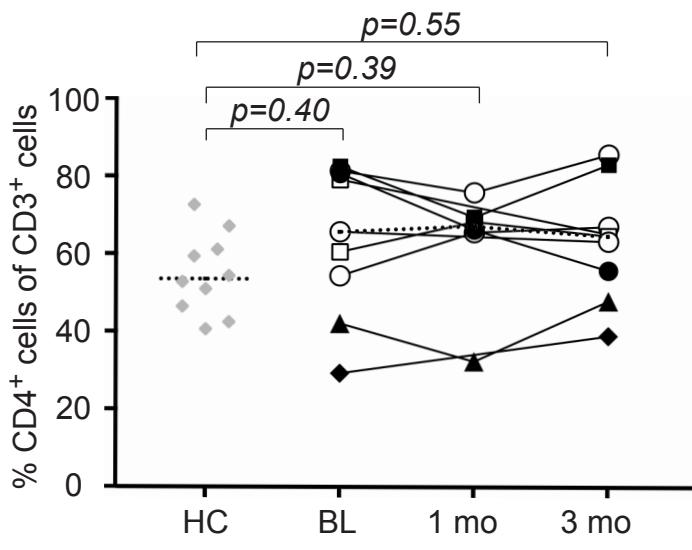
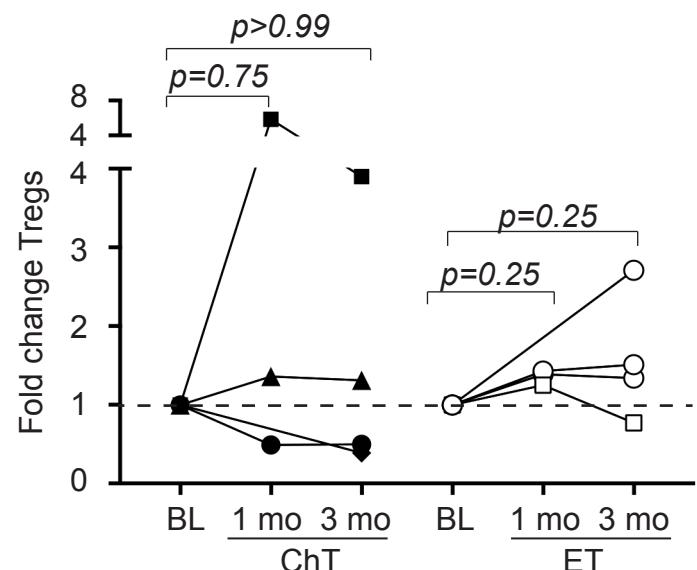
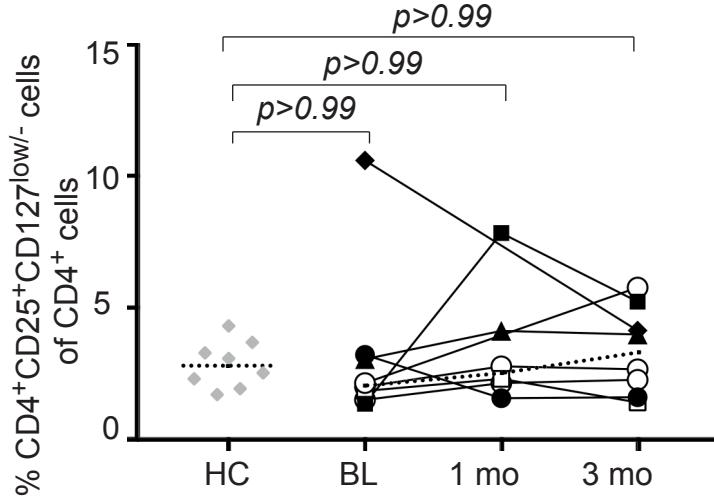
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Supplementary Figure 1.

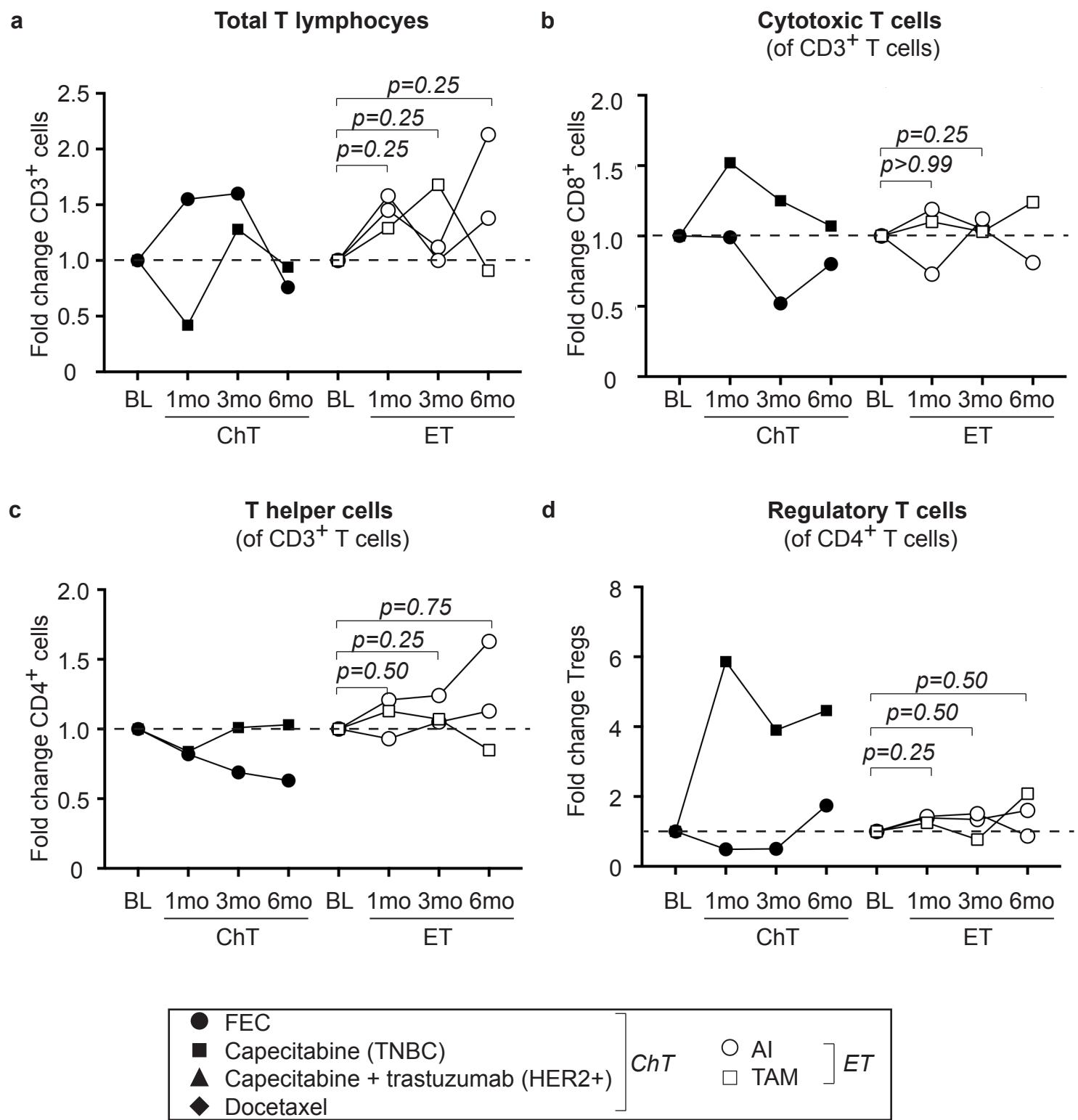


Supplementary Figure 2.

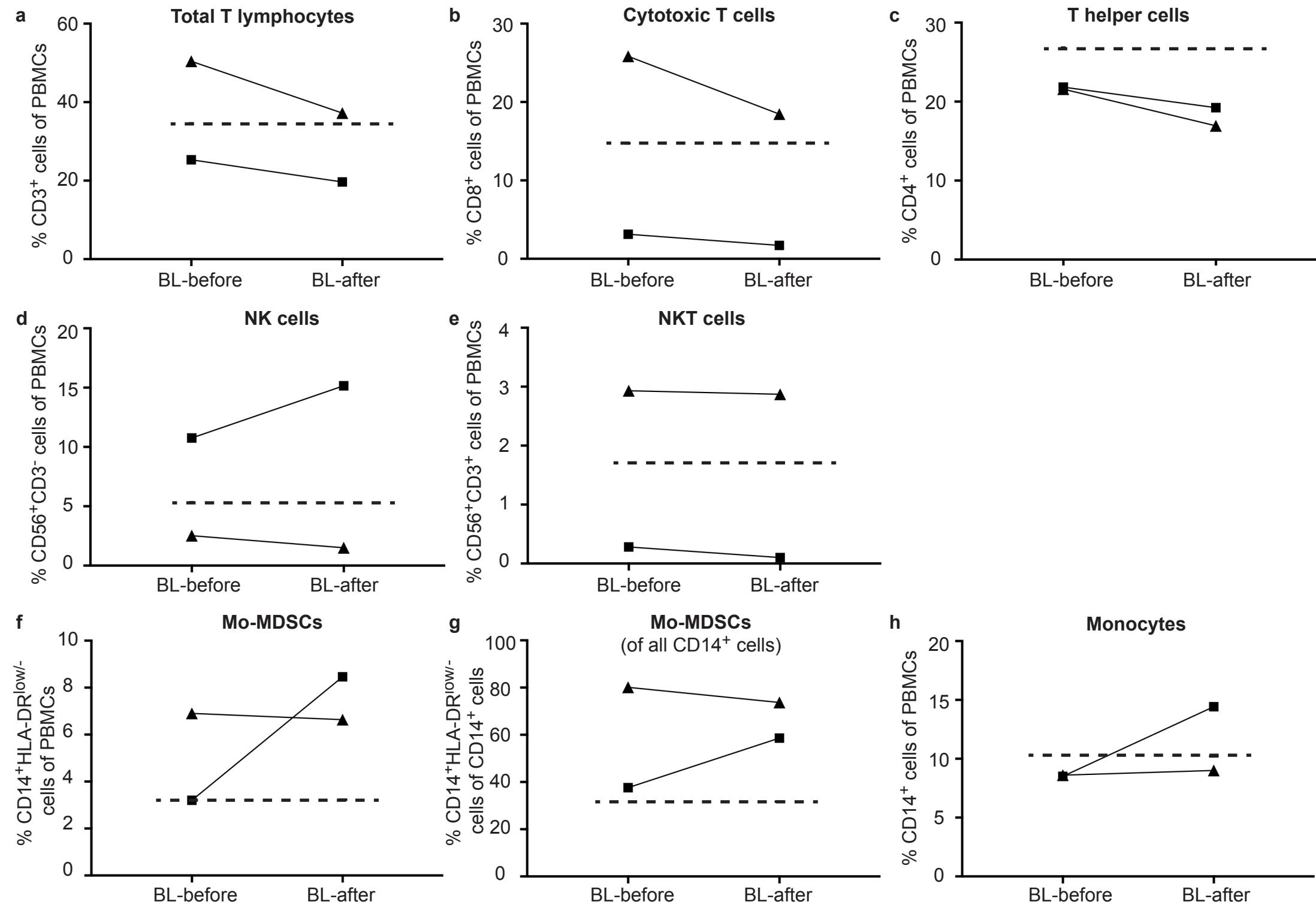
a**Cytotoxic T cells (of CD3⁺ T cells)****b****T helper cells (of CD3⁺ T cells)****c****Regulatory T cells (of CD4⁺ T cells)**

- FEC
 - Capecitabine (TNBC)
 - ▲ Capecitabine + trastuzumab (HER2+)
 - ◆ Docetaxel
- ChT*
○ AI
□ TAM
ET

Supplementary Figure 3.



Supplementary Figure 4.



Supplementary Figure Legends

Supplementary Figure 1. Representative dot plots and gating strategies of analyzed immune cell populations. All analyses are performed gated on viable (7AAD-negative) PBMCs. Percentage in gate shown. **a.** Total CD3⁺ T lymphocytes (black box) and CD8⁺ T_c/CTL (dashed box), **b.** CD4⁺ Th cells (black box) and CD4⁺CD25⁺CD127^{low/-} Tregs (dashed box). **c.** CD56⁺CD3⁻ NK cells (black box) and CD56⁺CD3⁺ NKT cells (dashed box). **d.** CD14⁺ monocytes (black box) and CD14⁺HLA-DR^{low/-} Mo-MDSCs (dashed box).

Supplementary Figure 2. Flow cytometric analyzes of peripheral blood mononuclear cells (PBMCs) from healthy controls (HC) and breast cancer patients before (baseline; BL), at 1 month or at 3 months of treatment. **(a-b, left panels)** Percentages of CD8⁺ T_c/CTLs (a) or CD4⁺ Th cells (b) of all CD3⁺ T lymphocytes, depicted over time for individual patients. Dashed lines represent median HC $n=10$, BL $n=9$, 1mo $n=6$ and 3mo $n=9$. Exact p-values, by Kruskal-Wallis with Dunn's multiple comparison test, are indicated. **(a-b, right panels)** Fold change as compared to BL of CD8⁺ T_c/CTLs (a) or CD4⁺ Th cells (b) upon treatment with chemotherapy (ChT, black symbols; BL $n=4$, 1mo $n=3$, 3mo $n=4$) or endocrine therapy (ET, white symbols; BL $n=5$, 1mo $n=3$, 3mo $n=5$). Dashed line represents BL levels (set to 1). Exact p-values, by Wilcoxon signed-rank test, are indicated. **(c, left panel)** Percentages of CD4⁺CD25⁺CD127^{low/-} Tregs of CD4⁺ cells depicted over time for individual patients. Dashed lines represent median. HC $n=10$, BL $n=8$ 1mo $n=6$ and 3mo $n=8$. Exact p-values, by Kruskal-Wallis with Dunn's multiple comparison test, are indicated. **(c, right panel)** Fold change of Tregs as compared to BL upon treatment with chemotherapy (ChT, black symbols) or endocrine therapy (ET, white symbols). Dashed line represents BL levels (set to 1). BL $n=4$, 1mo $n=3$, 3mo $n=4$. Exact p-values, by Wilcoxon signed-rank test, are indicated.

Supplementary Figure 3. Flow cytometric analyzes of peripheral blood mononuclear cells (PBMCs) from breast cancer patients before (baseline; BL), at 1 month, at 3 months or at 6 months of treatment with chemotherapy (ChT, black symbols, $n=2$) or endocrine therapy (ET, white symbols, $n=3$). Fold change of total CD3⁺ T lymphocytes of PBMCs (**a**), CD8⁺ T_c/CTLs of CD3⁺ cells (**b**), CD4⁺ Th of CD3⁺ cells (**c**) or Tregs of CD4⁺ cells (**d**) as compared to BL. Dashed line represent BL levels (set to 1). Exact p-values, by Wilcoxon signed-rank test are indicated.

Supplementary Figure 4. Flow cytometric analyzes of peripheral blood mononuclear cells (PBMCs) from breast cancer patients before and after radiation therapy, and before onset of chemotherapy. Percentages of total CD3⁺ T lymphocytes (**a**), CD8⁺ T_c/CTLs (**b**), CD4⁺ Th (**c**), CD56⁺CD3⁻ NK cells (**d**), CD56⁺CD3⁺ NKT cells (**e**), CD14⁺HLA-DR^{low/-} Mo-MDSCs (**f**), Mo-MDSCs of CD14⁺ cells (**g**) and CD14⁺ monocytes (**h**). Dashed lines represent median HC $n=10$.